Rising Sun Communications

J11-302173
(unexamined)

CAUTION Post-Edited
Machine Translation

(54) Title of the Invention

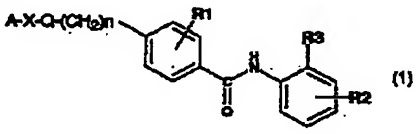
A histone deacetylase inhibiting agent

(Abstract) .

(Amended) .

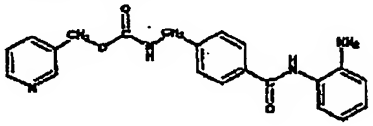
(Method of Solution)

The benzamide derivative represented by following general formula (1) having histone deacetylase inhibition action.



(1)

An example of the compound is as follows.



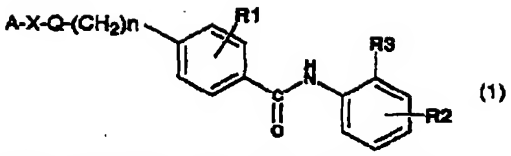
(effect) .

A benzamide derivative having the aforesaid histone deacetylase inhibition action is useful as therapy and/or improvement agent of disease involving proliferation of cell, effect enhancing drug of gene therapy or immunosuppressive drug. In particular effect as carcinostatic is high, and it is effective against hematopoietic organ tumour, solid cancer.

Patent Claims.

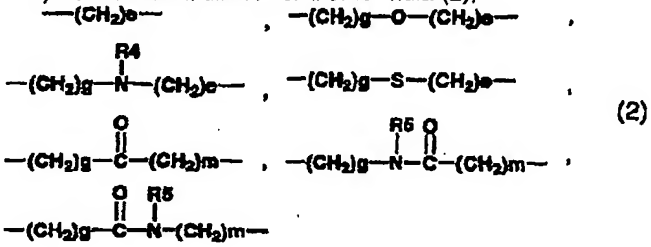
(Claim 1) .

A histone deacetylase inhibitor having as an active ingredient benzamide derivative represented by formula (1) or pharmacologically permitted salt thereof.



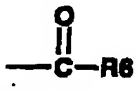
(1)

[In the formula, A denotes optionally substituted pyridine ring or condensed pyridine ring. (As the substituents, it has 1-4 groups selected from the group comprising halogen atom, hydroxy group, amino group, nitro group, cyano group, alkyl group of carbon number 1-4, alkoxy group of carbon number 1-4, amino alkyl group of carbon number 1-4, alkylamino group of carbon number 1-4, acyl group of carbon number 1-4, acylimino-group of carbon number 1-4, alkylthio group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, perfluoro alkyloxy group of carbon number 1-4, carboxyl group, alkoxycarbonyl group of carbon number 1-4). X denotes a direct bond or formula (2),



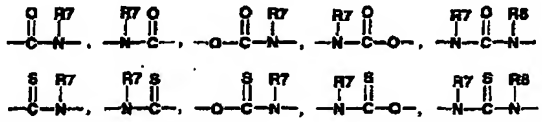
(2)

(In the formula, e denotes an integer of 1-4. The g and m respectively independently denote an integer of 0-4. R4 denotes any of the structures represented by hydrogen atom, optionally substituted alkyl group of carbon number 1-4 or an acyl group represented by formula (3),



(3)

(In the formula, R6 denotes acyl group represented by optionally substituted alkyl group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, phenyl group or pyridine ring). R5 denotes hydrogen atom or optionally substituted alkyl group of carbon number 1-4, n denotes an integer of 1-4. Q denotes any of structures represented by formula (4),

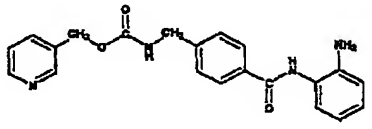


(4)

(In the formula, R7 and R8 respectively independently denote hydrogen atom or optionally substituted alkyl group of carbon number 1-4). R1 and R2 respectively independently denote hydrogen atom, halogen atom, hydroxy group, amino group, alkyl group of carbon number 1-4, alkoxy group of carbon number 1-4, amino alkyl group of carbon number 1-4, alkylamino group of carbon number 1-4, acyl group of carbon number 1-4, acylimino-group of carbon number 1-4, alkylthio group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, perfluoro alkyloxy group of carbon number 1-4, carboxyl group or alkoxycarbonyl group of carbon number 1-4. R3 denotes amino group or hydroxy group.]

(Claim 2) .

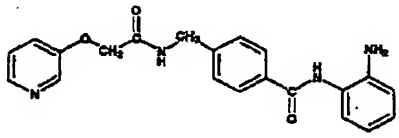
A histone deacetylase inhibitor having as an active ingredient a benzamide derivative represented by formula (5) or pharmacologically permitted salt thereof.



(5)

(Claim 3) .

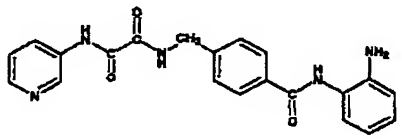
A histone deacetylase inhibitor having as an active ingredient a benzamide derivative represented by formula (6) or pharmacologically permitted salt thereof.



(6)

(Claim 4) .

A histone deacetylase inhibitor having as an active ingredient a benzamide derivative represented by formula (7) or pharmacologically permitted salt thereof.



(7)

(Claim 5) .
A carcinostatic containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 6) .
A skin disease therapy and/or improvement agent containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 7) .
A therapy and/or improvement agent of infection containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 8) .
A therapy and/or improvement agent of allergic disease containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 9) .
A therapy and/or improvement agent of autoimmune disease containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 10) .
A gene therapy effect promoter containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 11) .
A therapy and/or improvement agent of vascular disease containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

(Claim 12) .
A drug containing as effective component at least one inhibitor in accordance with any of Claims 1-4.

Detailed Description.
[Detailed Description of the Invention]

(0001)

(Technical Sphere of this Invention) .

This invention relates to a benzamide derivative having histone deacetylase inhibition action. More particularly this invention relates to the used as carcinostatic and other drug on the basis of histone deacetylase inhibition action.

(0002)

(technology of the prior art)

DNA forms complex with histone in nucleus of cell, and chromatin structure folded in high orders is formed, and it is held at inert condition (Knezetic et al., Cell, 45: 95-104, 1986 and the like). When genetic transcription is performed in nucleus, it is required that the structure thereof is derived into unwound condition so that various transcription factors can be contacted with DNA (Felsenfeld et al., Cell, 86: 13-19, 1996). The relationship between acetylation of histone and activation of transcription has been reported in the past, but it has become clear that one of the actions to cause a change in the structure leading to transcription activation was acetylation of histone (Hong et al., J. Biol. Chem., 268: 305-314, 1993 and the like). Moreover, histone acetylation enzyme (histone acetyl transferase) and histone deacetylation enzyme (histone deacetylase, HDA) are ones controlling acetylation thereof and importance thereof has been recently recognised (A. Csordas, Biochem. J., 265: 23, 1990 and the like). Sodium butyrate with which arrest of cell cycle and induction of differentiation had been confirmed for a long time is a representative HDA inhibitor (L.S. Cousens et al., J. Biol. Chem., 254: 1716, 1979 and the like), and has clinical use has also been tried (Novogrodsky et al., Cancer, 51: 9-14, 1983 and Miller et al., Eur. J. Cancer Clin. Oncl 23: 1283-1287, 1987). However, because fundamental inhibiting activity was low and in-vivo sustainability was also short, a high dosage was required to demonstrate effect. Therefore increase of sustainability has been attempted with a prodrug of butyric acid (Zi-Xing et al. Cancer. Res., 54: 3494-3499, 1994 and Kasukabe et al., British J. Cancer, 75(6): 850-854, 1997 and the like).

(0003)

Moreover, a natural product, trichostatin A (TSA) was found to derive arresting of cell cycle (Yoshida et al., Exp. Cell Res., 177: 122-131, 1988), proliferation stop, induction of differentiation (Yoshida et al., cancer Res., 47: 3688-3691, 1987), induction of cell

morphology change and apotosis. As the mechanism thereof, TSA was confirmed to be a HDA inhibitor having high activity in vitro (Yoshida et al., J. Biol. Chem., 265: 17174, 1990).

(0004)
Moreover, studies of other HDA inhibitors have been continued, and an inhibitory action has been found in trapoxin(?) (Itazaki et al., J. Antibiot., 43(12): 1524-1534, 1990 and the like), phenylbutyric acid (Carducci et al., Clin. Cancer Res., 2(2): 379, 1996 and the like) and so forth. As those HDA inhibitors have cell cycle arrest and differentiation induction actions, application is anticipated primarily as a carcinostatic. Moreover, in addition, as far as HDA inhibitors are concerned, application is anticipated in various drugs.

(0005)
In other words, as a therapy / improvement drug of diseases involving a proliferation of cell, various applications such as for example therapy / improvement drug for autoimmune disease, dermatopathia, infection (Darkin- Rattray et al., Proc. Natl. Acad. Sci. USA, 93: 13143-13147, 1996), moreover more efficient introduction of vector in gene therapy (Dion et al., Virology, 231: 201-209, 1997), expression facilitation of transgene (Chen et al., Proc. Natl. Acad. Sci. USA, 94: 5798-5803, 1997) have been attempted. However, the inhibitors so far have not reached the level that are thoroughly satisficatory for a drug when stability, toxicity, drug kinetics or active strength are considered. So development of a drug which solved those problems is strongly desired.

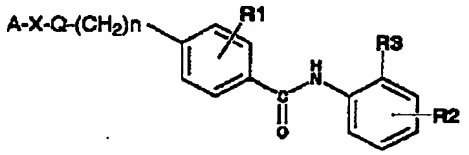
(0006)
[Problems to be Overcome by this Invention]
The object of this invention is to put forward a compound useful as drug such as effect potentiation drug of gene therapy with improved problems of HDA inhibitor of prior art and which is useful as therapy and/or improvement agent of diseases involving proliferation of cell.

(0007)
[Means to Overcome these Problems]
These inventors carried out assiduous investigations to solve these problems, and as a result confirmed that benzamide derivative already reported to have differentiation induction

action (JP Patent Application 09-260277) had strong HDA inhibitory effect. This invention was completed on the basis of this discovery.

(0008)
In other words this invention is [1] a histone deacetylase inhibitor having as an active ingredient benzamide derivative represented by formula (1) or pharmacologically permitted salt thereof.

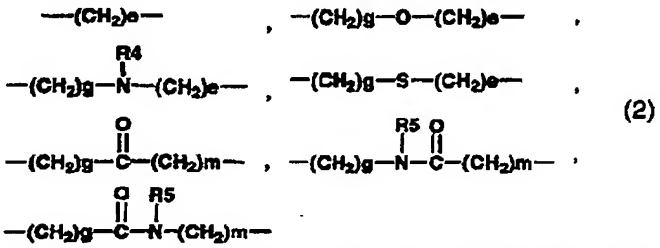
(0009)



(1)

[In the formula, A denotes optionally substituted pyridine ring or condensed pyridine ring. (As the substituents, It has 1-4 groups selected from the group comprising halogen atom, hydroxy group, amino group, nitro group, cyano group, alkyl group of carbon number 1-4, alkoxy group of carbon number 1-4, amino alkyl group of carbon number 1-4, alkylamino group of carbon number 1-4, acyl group of carbon number 1-4, acylimino-group of carbon number 1-4, alkylthio group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, perfluoro alkyloxy group of carbon number 1-4, carboxyl group, alkoxycarbonyl group of carbon number 1-4). X denotes a direct bond or formula (2),

(0010)



(2)

{In the formula, e denotes an integer of 1-4. The g and m respectively independently denote an integer of 0-4. R4 denotes any of the structures represented by hydrogen atom, optionally substituted alkyl group of carbon number 1-4 or an acyl group represented by formula (3),

(0011)

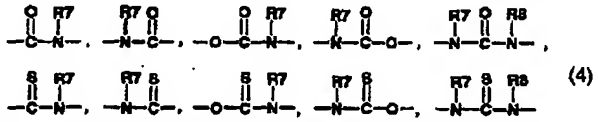
(3)

(In the formula, R6 denotes acyl group represented by optionally substituted alkyl group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, phenyl group or pyridine ring). R5 denotes hydrogen atom or optionally substituted alkyl group of carbon number 1-4, n denotes an integer of 1-4.

(0012)

Q denotes any of structures represented by formula (4),

(0013)



(4)

(In the formula, R7 and R8 respectively independently denote hydrogen atom or optionally substituted alkyl group of carbon number 1-4).

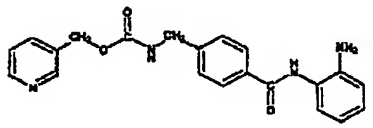
(0014)

R1 and R2 respectively independently denote hydrogen atom, halogen atom, hydroxy group, amino group, alkyl group of carbon number 1-4, alkoxy group of carbon number 1-4, amino alkyl group of carbon number 1-4, alkylamino group of carbon number 1-4, acyl group of carbon number 1-4, acylimino-group of carbon number 1-4, alkylthio group of carbon number 1-4, perfluoro alkyl group of carbon number 1-4, perfluoro alkyloxy group of carbon number 1-4, carboxyl group or alkoxycarbonyl group of carbon number 1-4.

(0015)

R3 denotes amino group or hydroxy group.], moreover it is [2] a histone deacetylase inhibitor having as an active ingredient benzamide derivative represented by formula (5)

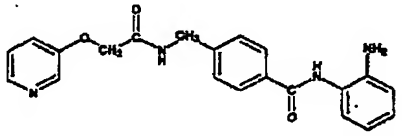
(0016)



(5)

or pharmacologically permitted salt thereof, moreover it is [3] a histone deactylase inhibitor having as an active ingredient benzamide derivative represented by formula (6)

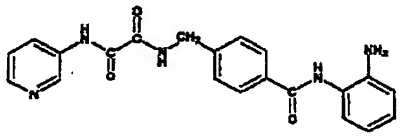
(0017)



(6)

or pharmacologically permitted salt thereof, moreover it is [4] a histone deacetylase inhibitor having as an active ingredient benzamide derivative represented by formula (7)

(0018)



(7)

or pharmacologically permitted salt thereof, moreover it is [5] an carcinostatic containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [6] a skin disease therapy and/or improvement agent containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [7] a therapy and/or improvement agent of infection containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [8] a therapy and/or improvement agent of allergic disease containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [9] a therapy and/or improvement agent of autoimmune disease containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [10] a gene therapy effect promoter containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [11] a therapy and/or improvement agent of vascular disease containing as effective ingredient at least one member in accordance with any of [1]-[4], moreover it is [12] a drug containing as effective ingredient at least one member in accordance with any of [1]-[4].

(0019)

[Conditions for carrying out this invention]

Hereinafter this invention is described in greater detail. As carbon number 1-4 stated in this invention, the carbon number per the unit substituent is denoted. In other words, in case of for example dialkyl substitution the carbon number 2-8 is denoted.

(0020)

As condensed pyridine ring in the compound represented by formula (1), bicyclic condensed pyridine ring such as quinoline, isoquinoline, naphthyridine, furopyridine, thienopyridine, pyrrolopyridine, oxazolo pyridine, imidazolo pyridine, thiazolopyridine are nominated. As halogen atom, fluorine atom, chlorine atom, bromine atom, iodine atom are nominated.

(0021)

As alkyl group of carbon number 1-4, for example methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, sec-butyl group, t-butyl group are nominated. As alkoxy group of carbon number 1-4, for example methoxy group, ethoxy group, n-propoxy group, isopropoxy group, allyloxy group, n-butoxy group, isobutoxy group, sec-butoxy group, t-butoxy group are nominated. As amino alkyl group of carbon number 1-4, for example aminomethyl group, 1-amino ethyl group, 2-aminopropyl group are nominated.

(0022)

As alkylamino group of carbon number 1-4, for example N-methylamino group, N,N-dimethylamino group, N,N-diethylamino group, N-methyl-N-ethylamino group, N,N-diisopropylamino group are nominated. As acyl group of carbon number 1-4, for example acetyl group, propanoyl group, butanoyl group are nominated.

(0023)

As acylimino-group of carbon number 1-4, for example acetylamino group, propanoyl amino group, butanoyl amino groups are nominated.

(0024)

As alkylthio group of carbon number 1-4, methylthio group, ethylthio group, propylthio group are nominated. As perfluoro alkyl group of carbon number 1-4, for example trifluoromethyl group, pentafluoro ethyl groups are nominated.

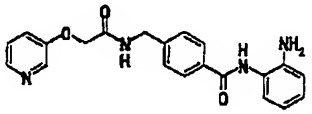
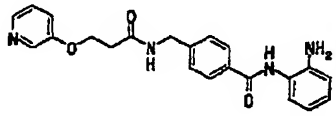
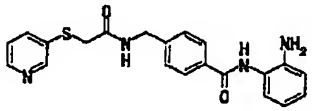
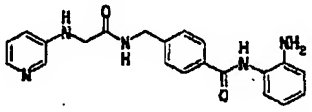
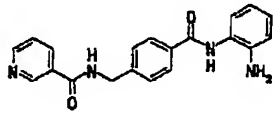
(0025)
As perfluoro alkyloxy group of carbon number 1-4, for example trifluoro methoxy group, pentafluoro ethoxy group are nominated. As alkoxycarbonyl group of carbon number 1-4, for example methoxycarbonyl group, ethoxycarbonyl group are nominated.

(0026)
As optionally substituted alkyl group of carbon number 1-4, for example methyl group, ethyl group, n-propyl group, isopropyl group, n-butyl group, isobutyl group, sec-butyl group, t-butyl group and one containing 1-4 groups selected from the group comprising halogen atom, hydroxy group, amino group, nitro group, cyano group, phenyl group, pyridine ring as the substituent to this are nominated. As salt of the pharmacologically permitted compound, salt of inorganic acid such as hydrochloric acid, hydrobromic acid, sulphuric acid, orthophosphoric acid used regularly in this sphere, and salt of organic acid such as acetic acid, lactic acid, tartaric acid, malic acid, succinic acid, fumaric acid, maleic acid, citric acid, benzoic acid, trifluoroacetic acid, p-toluenesulfonic acid, methanesulfonic acid are nominated.

(0027)
The drug denotes therapy and/or improvement drug for such as dermatopathia, infection, allergic disease, autoimmune disease, vascular disease or gene therapy effect promoter in addition to carcinostatic. In the compound represented by formula (1), when asymmetric carbon is present, it can be present in a form of mixture of stereoisomerism form including different stereoisomerism form or the racemic form. In other words this invention includes various kinds of forms prescribed like these, but these can be used as the effective ingredient compound in the same way.

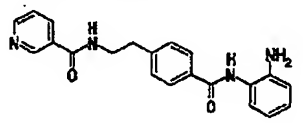
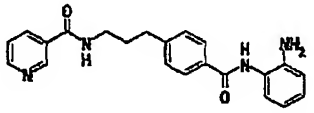
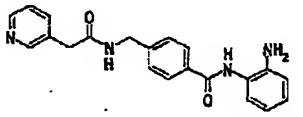
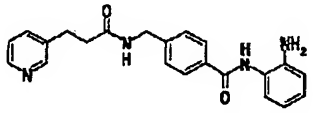
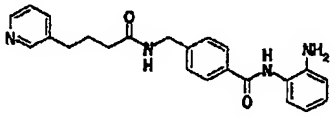
(0028)
Hereinafter typical compounds represented by formula (1) of this invention are exemplified in Table-1 (Table 1 - Table 14). Moreover this invention is not restricted to these examples.

(0029)
(Table 1).

compound No.	Structural formula
1	
2	
3	
4	
5	

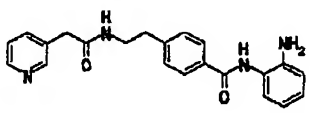
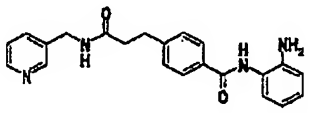
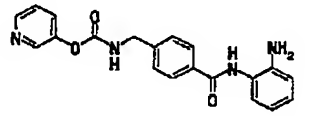
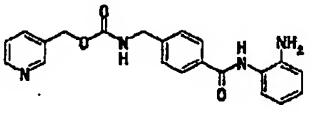
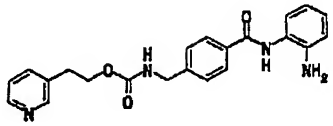
(0030)

(Table 2) .

compound No.	Structural formula
6	
7	
8	
9	
10	

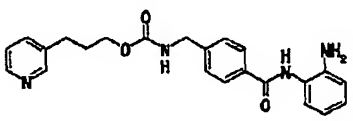
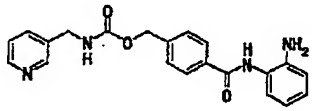
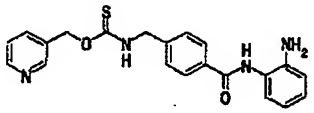
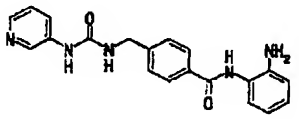
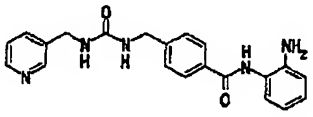
(0031)

(Table 3) .

compound No.	Structural formula
11	
12	
13	
14	
15	

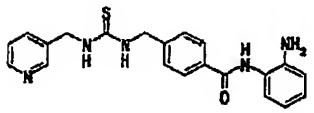
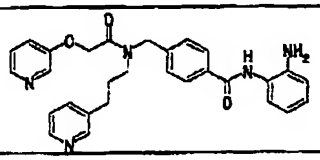
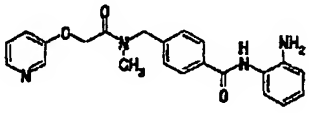
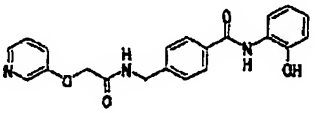
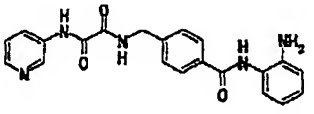
(0032)

(Table 4) .

compound No.	Structural formula
16	
17	
18	
19	
20	

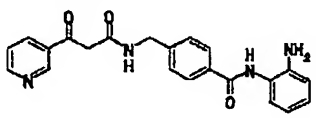
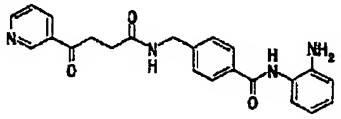
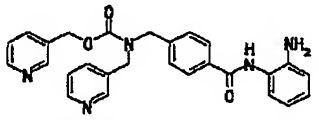
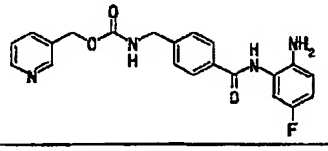
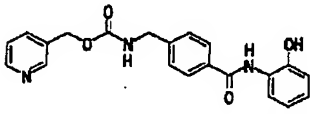
(0033)

(Table 5) .

compound No.	Structural formula
21	
22	
23	
24	
25	

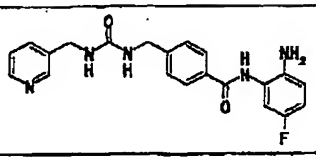
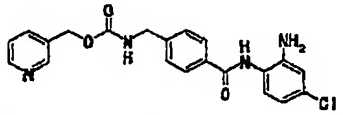
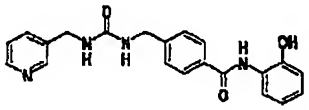
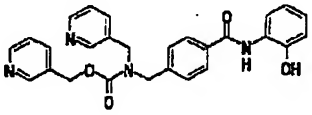
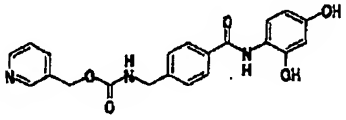
(0034)

(Table 6) .

compound No.	Structural formula
26	
27	
28	
29	
30	

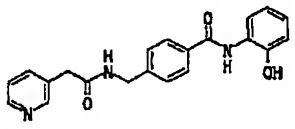
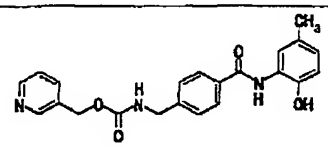
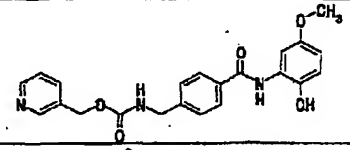
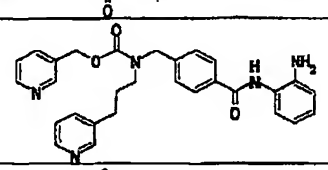
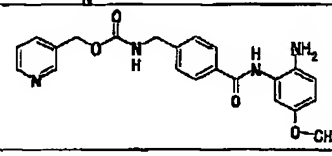
(0035)

(Table 7) .

compound No.	Structural formula
31	
32	
33	
34	
35	

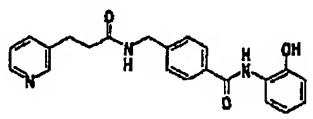
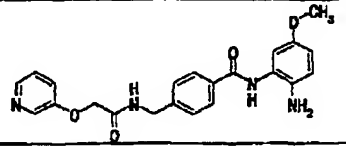
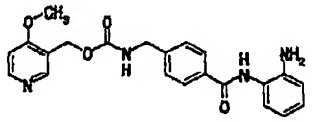
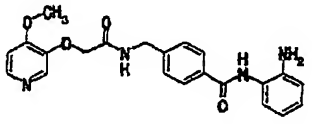
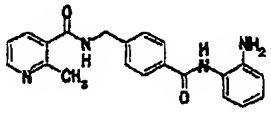
(0036)

(Table 8) .

compound No.	Structural formula
36	
37	
38	
39	
40	

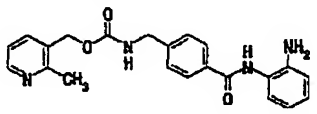
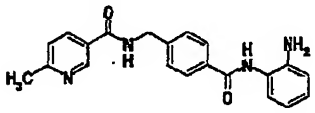
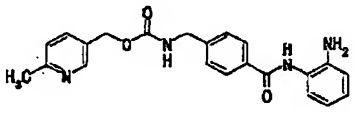
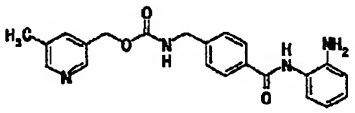
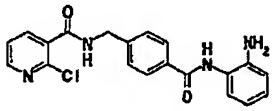
(0037)

(Table 9) .

compound No.	Structural formula
41	
42	
43	
44	
45	

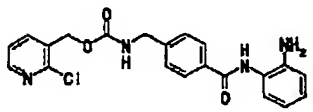
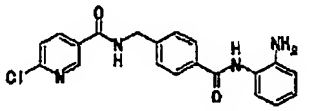
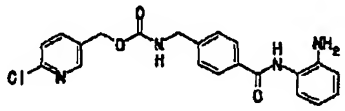
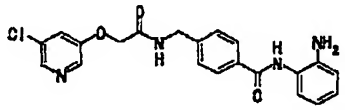
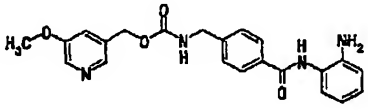
(0038)

(Table 10) .

compound No.	Structural formula
46	
47	
48	
49	
50	

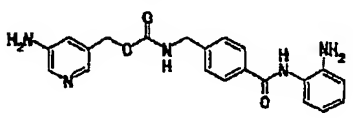
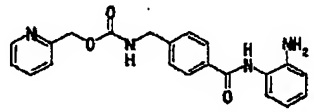
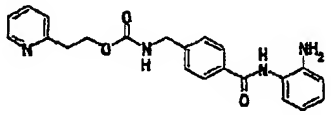
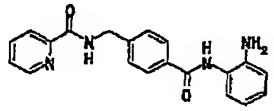
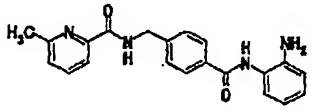
(0039)

(Table 11) .

compound No.	Structural formula
51	
52	
53	
54	
55	

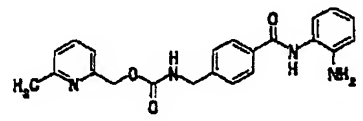
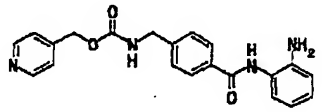
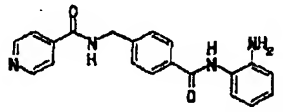
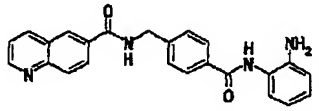
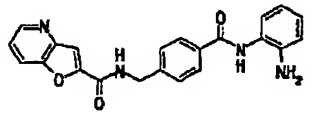
(0040)

(Table 12) .

compound No.	Structural formula
56	
57	
58	
59	
60	

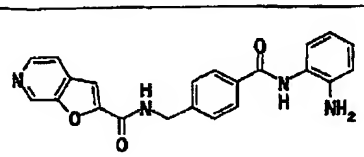
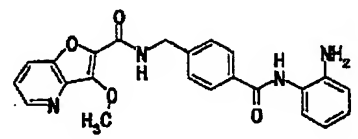
(0041)

(Table 13) .

compound No.	Structural formula
61	
62	
63	
64	
65	

(0042)

(Table 14) .

compound No.	Structural formula
66	
67	

Manufacturing of the compound represented by formula (1) of this invention or pharmacologically acceptable salts thereof can be carry out by process in accordance with Japanese Patent Application No. 09-260277, but can be produced by for example the following process.

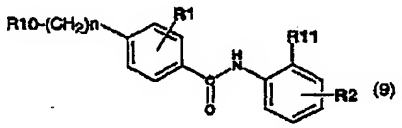
[a] The compound represented by formula (8) .

(0043)

$$A\text{-}X\text{-}R9 \qquad (8)$$

[in the formula, A and X has the same said meanings. R9 denotes -C(=G)OH (G denotes oxygen atom or sulphur atom) or -NH2] and the compound represented by formula (9)

(0044)



(9)

[in the formula, R1, R2 and n have the same said meanings. When R9 denotes -C =G)OH (G denotes same said meanings) R10 denotes -NH2, and when R9 denotes -NH2, R10 denotes C =G)OH (G denotes same said meanings). R11 denotes a protected amino group protected by protecting group used by ordinary peptide forming reaction such as t-

butoxycarbonyl group, or a protected hydroxy group protected by protecting group used ordinary peptide forming reaction such as benzyl group] are subjected to condensation reaction, or

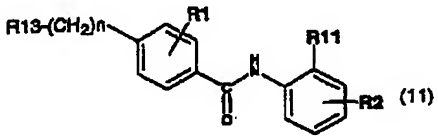
[b] The compound represented by formula (10)

(0045)

$$A\text{-}X\text{-}R12 \quad (10)$$

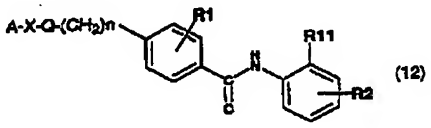
(in the formula, A and X has the same said meanings. R12 denotes -OH or -NH2) and the compound represented by formula (11)

(0046)



(in the formula, R1, R2, R11 and n have the same said meanings. R13 denotes -OH or -NH2) are subjected to condensation reaction using N,N-carbonyldiimidazole, N,N-thiocarbonyl diimidazole, phosgene or thiophosgene, and it is possible to obtain the compound of this invention by eliminating protecting group of the thereby obtained compound represented by formula (12)

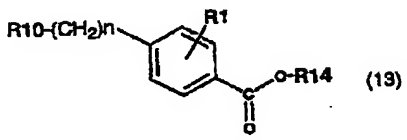
(0047)



(in the formula, A, X, Q, n, R1, R2 and R11 have the same said meanings).

[c] The compound represented by formula (8) and the compound represented by formula (13)

(0048)

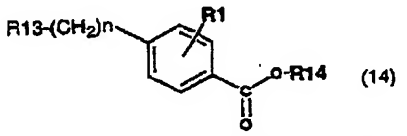


(13)

(In the formula, R1, R10 and n have the same said meanings. R14 denotes methyl group ethyl group or tert-butyl group.) are subjected to condensation reaction, or

[d] The compound represented by formula (10) and the compound represented by formula (14)

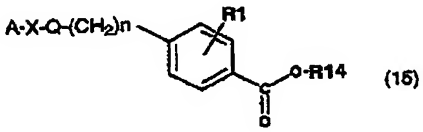
(0049)



(14)

(In the formula, R1, R13, R14 and n have the same said meanings) are subjected to condensation reaction using N,N-carbonyldiimidazole, N,N-thiocarbonyl diimidazole, phosgene or thiophosgene, thereby obtained compound represented by formula (15)

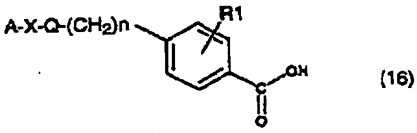
(0050)



(15)

(in the formula, A, X, Q, n, R1 and R14 have the same said meanings) is hydrolysed, thereby obtained compound represented by formula (16)

(0051)

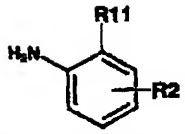


(16)

(in the formula, A, X, Q, n, and R1 have the same said meanings)

and the compound represented by formula (17)

(0052)

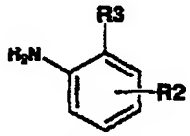


(17)

(in the formula, R2 and R11 have the same said meanings) are subjected to condensation reaction, and it is possible to obtain the compound of this invention by eliminating protecting group of the thereby obtained compound represented by formula (12).

[e] It is possible to obtain the compound of this invention by subjecting the compound represented by formula (16) and the compound represented by formula (18)

(0053)



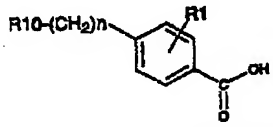
(18)

(in the formula, R2 and R3 have the same said meanings) to condensation reaction.

(0054)

Synthesis of typical intermediates is described. The compound represented by formula (8) can be obtained by introducing suitable protecting group to the benzoic acid derivative represented by formula (19)

(0055)



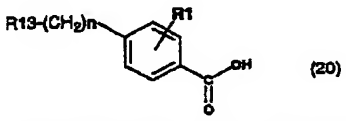
(19)

(in the formula R1, R10 and n have the same said meanings) thereafter subjecting to condensation reaction with the compound represented by formula (17), and furthermore carrying out deprotection.

(0056)

The compound represented by formula (11) can be obtained by introducing suitable protecting group to benzoic acid derivative represented by formula (20)

(0057)



(20)

(in the formula R1, R13 and n have the same said meanings) and thereafter subjecting to condensation reaction with the compound represented by formula (17) and furthermore carrying out deprotection. The compound represented by formula (17) can be obtained by introducing protecting group to the compound represented by formula (18).

(0058)
Next, the reaction is described.

Condensation reaction of [a] can be put into effect by amide bond forming reaction in ordinary peptide, for example process of active ester or mixed acid anhydride or acid chloride. The compound represented by for example carboxylic acid component [a compound wherein R9 is -C(=G)OH (G has the same said meanings) in formula (8), or a compound wherein R10 is -C(=G)OH (G has the same said meanings) in formula (9)] and phenols such as 2,4,5-trichlorophenol, pentachlorophenol or 4-nitrophenol and the like, or N-hydroxy compound such as N-hydroxysuccinimide, N-hydroxybenzotriazole and the like are condensed in the presence of dicyclohexylcarbodiimide, and converted into active ester form, thereafter it can be carried out by condensing with amine component [a compound wherein R9 is -NH2 in formula (8) or a compound wherein R10 is -NH2 or formula (9)].

(0059)
Moreover, carboxylic acid component [a compound wherein R9 is -C(=G)OH (G has the same said meanings) in formula (8), or a compound wherein R10 is -C(=G)OH (G has the same said meanings) in formula (9)] is reacted with oxalyl, thionyl chloride, phosphorus oxychlorides chloride, thereby it is converted to acid chloride, and thereafter it can be carried out by condensing with amine component [a compound wherein R9 is -NH2 in formula (14) or a compound wherein R10 is -NH2 or formula (9)].

(0060)
Moreover, carboxylic acid component [a compound wherein R9 is -C(=G)OH (G has the same said meanings) in formula (8), or a compound wherein R10 is -C(=G)OH (G has the same said meanings) in formula (9)] is reacted with isobutyl chlorocarbonate or methanesulphonyl chloride, thereby a mixed acid anhydride is obtained, thereafter it can be carried out by condensing with amine component [a compound wherein R9 is -NH2 in formula (8) or a compound wherein R10 is -NH2 or formula (9)].

(0061)
Furthermore, aforesaid condensation reaction can be performed by using single species of peptide condensation reagent such as dicyclohexylcarbodiimide, N,N-carbonyldiimidazole, diphenyl phosphoric acid azide, diethyl phosphoric acid cyanide, 2-chloro-1,3-dimethyl imidazolo aluminum chloride.

(0062)
Reaction is carried out at -20 to +50 degrees usually for 30 minutes to 48 hours. As solvent used, for example, an alcohol such as methanol, methanol and the like or a mixture thereof are nominated in addition to aromatic hydrocarbon species such as benzene, toluene, ethers such as tetrahydrofuran, dioxane, diethyl ether and the like, halogenated hydrocarbons such as methylene chloride, chloroform, N,N-dimethylformamide. In accordance with requirements organic base, for example triethylamine or pyridine is added and is reacted.

(0063)
Condensation reaction of [b] can be carried out by activating either of the compound represented by formula (10) or formula (11) using phosgene, thiophosgene, N,N-carbonyldiimidazole and N,N'-thiocarbonyl diimidazole and the like, and thereafter reacting with the compound of the other. Reaction is performed usually at -20 to +50 degrees for 30 minutes to 48 hours. As solvent used, for example, aromatic hydrocarbon species such as benzene, toluene, ethers such as tetrahydrofuran, dioxane, diethyl ether and the like, halogenated hydrocarbons such as methylene chloride, chloroform, N,N-dimethylformamide or mixture thereof are nominated. In accordance with requirements organic base, for example triethylamine or pyridine is added and is reacted.

(0064)
Condensation reaction of [c] can be performed by process same as in condensation reaction of [a].

(0065)
Condensation reaction of [d] can be performed by process same as in condensation reaction of [b]. Elimination of protecting groups of the compound represented by formula (11) is performed under conditions used by ordinary peptide forming reaction. For example, when R11 in formula (12) is an amino group protected with t-butoxycarbonyl group, it is possible to carry out deprotecting reaction by being treating with hydrochloric acid or acid such as trifluoroacetic acid.

(0066)
Salt of the compound represented by formula (1) can be obtained by reaction to produce compound represented by formula (1), but the salt can be easily formed with pharmacologically acceptable acid. As acid thereof, for example inorganic acid such as hydrochloric acid, hydrobromic acid, sulphuric acid, orthophosphoric acid and organic acid such as acetic acid, tartaric acid, fumaric acid, maleic acid, citric acid, benzoic acid, trifluoroacetic acid, p-toluenesulfonic acid are nominated. These salts can also be used as the effective ingredient compound of this invention in the same way as in the free form of the compound of formula (1).

(0067)
The compound represented by formula (1) can be isolated and purified from the reaction mixture by ordinary separation means, for example process such as extraction, recrystallisation method, column chromatography.

(0068)
Benzamide derivative having histone deacetylase inhibition action of this invention is useful as therapy and/or improvement agent of disease involving proliferation of cell, effect potentiation drug of gene therapy or immunosuppressive drug. Wherein, the disease involving proliferation of cell, malignant tumour, autoimmune disease, dermatopathia, infection, vascular disease, allergic disease, gastrointestinal tract injury, hormonal disease, diabetes mellitus are nominated.

(0069)
As malignant tumour, in addition to hematopoietic organ tumours such as acute leukaemia, chronic leukaemia, malignancy lymphoma, multiple myeloma, macroglobulinemia, solid tumours such as colon cancer, brain tumour, head cervix cancer, breast cancer, lung cancer, cancer of the esophagus, gastric cancer, hepatoma, gallbladder cancer, bile duct cancer, pancreatic carcinoma, insula pancreatica cell cancer, kidney cell cancer, adrenal cortex cancer, tumour of the urinary bladder, prostatic cancer, testis tumour, ovary cancer, uterine cancer, carcinoma villosum, cancer of the thyroid, bad carcinoid tumour, skin cancer, malignant melanoma, osteosarcoma, soft tissue sarcoma, neuroblastoma, Wilms tumour, retinoblastoma are nominated. As autoimmune disease, rheumatism, nephritis, diabetes mellitus, systemic lupus erythematosus, human autoimmune lymphocytotic lymphadenopathy, immunoblastic lymphadenopathy, Crohn's disease, ulcerative colitis are nominated. As dermatopathia, psoriasis, acne, eczema, atopic dermatitis, parasitic dermatosis, alopecia, pyogenic dermatosis, skin sclerosis are nominated. As infection, diseases caused by infection of such as various bacteria, viruses or parasites are denoted. As vascular disease, arteriosclerosis and the like are nominated. As effect potentiation of gene therapy, more efficient introduction of gene vector, expression facilitation of transgene are nominated. Moreover subject disease of this invention is not necessarily restricted to these.

(0070)
The effective ingredient compounds of this invention are useful as drug, and these are used in a form of general medical formulation. Formulation is prepared using diluent of for example filler, expander, binding agent, moisturizing agent, disintegrating agent, surface active agent, lubricant or excipient which are usually used. As this drug formulation, various forms can be selected corresponding to the therapy object and as representative thereof, tablet, pill, powder, liquid medicine, suspending agent, emulsion, granule, capsule agent, injection (liquid medicine, suspending agent) and suppository and the like are nominated.

(0071)
When it is formed into a tablet, various ones which is known well in the prior art as a carrier in this sphere, can be widely used. As example thereof, for example excipient such as lactose, dextrose, starch, calcium carbonate, kaolin, crystalline cellulose, silicic acid, and

the like, binding agent such as water, ethanol, propyl alcohol, single syrup, dextrose liquid, starch liquid, gelatin solution, carboxymethyl cellulose, shellac, methyl cellulose, polyvinylpyrrolidone and the like, disintegrating agent such as dried starch, sodium alginate, agar powder, carmellose calcium, starch, lactose, disintegration depressant such as refined sugar, cacao butter, hydrogenation oil, absorption promoter such as quaternary ammonium salt group, sodium lauryl sulphate and the like, moisturising agent such as glycerin, starch and the like, adsorbent such as starch, lactose, kaolin, bentonite, colloidal silicic acid, lubricant such as talc, stearate, polyethyleneglycol and the like can be used. Furthermore, as for a tablet, it can be made into coated tablet of ordinary agent in accordance with requirements, for example sugar coated tablet, gelatin encapsulation tablet, enteric-coated encapsulation tablet, film coating tablet or bilayer tablet, multilayer tablet.

(0072)
When it is formed into pill, ones well known in prior art in this sphere as a carrier, can be widely used. As example thereof, for example excipients such as crystalline cellulose, lactose, starch, hardening vegetable oil, kaolin, talc and the like, binding agent such as powdered gum Arabic, tragacanth powder, gelatin and the like, disintegrating agent such as carmellose calcium, agar and the like are nominated.

(0073)
Capsule agent is prepared by mixing the effective ingredient compound with above-mentioned various carriers according to conventional method, and packing into hard gelatin capsule, soft capsule and the like.

(0074)
When it is prepared as injection, it is preferred that liquid medicine, emulsion and suspending agent are sterilised and are isotonic with blood, and when it is formed into these, ones conventionally used in prior art in this sphere as diluent, for example water, ethanol, macrogol, propylene glycol, ethoxylation isostearyl alcohol, polyoxyisosteary alcohol, polyoxyethylene sorbitan fatty acid ester species can be used. In this case, sodium chloride, dextrose or glycerin of necessary quantity may be contained in drug formulation to prepare an isotonic solution, and moreover ordinary solubiliser, buffer agent, analgesic and the like may be added.

(0075)
When it is formed into suppository, ones well known in prior art as a carrier can be widely used. As example thereof, for example semi-synthetic glyceride, cacao butter, esters of higher alcohol, higher alcohol, polyethyleneglycol and the like are nominated.

(0076)
Furthermore colorant, preservative, flavour, flavour agent, sweetener and other drug can be contained in the drug formulation in accordance with requirements.

(0077)
The quantity of the effective ingredient compound which should be contained in these drug formulations of this invention is not restricted in particular and suitably selected from a wide range, but it is usually about 1-70 wt.% and preferably made into about 5-50 wt.% in the formulation composition.

(0078)
As for the administration method of these drug formulation of this invention, there are no restrictions in particular and it is administered by the methods that suits various formulation, age of patient, sex, degree of disease and other conditions. For example, in the cases of tablet, pill, liquid medicine, suspending agent, emulsion, granule and capsule agent, it is orally-administered, and in the case of injection, it is administered intravenously by itself or by being mixed with ordinary fluid replacement such as glucose, amino acid, and furthermore it is administered intramuscularly, subcutaneously or intraperitoneously by itself in accordance with requirements. In the case of suppository, it is administered in rectum.

(0079)
Dosage of these drug formulation of this invention is suitably selected by application, age of patient, sex, degree of disease and other conditions, but it is usually made into about around 0.0001-100 mg as the quantity of the effective ingredient compound per day per 1 kg weight. Moreover, it is desirable that the effective ingredient compound is contained by about 0.001-1,000 mg range in the formulation of administration unit form.

(0080)

The compound and salts thereof represented by formula (1) of this invention do not demonstrate toxicity in dosage that demonstrates pharmacological effect.

(0081)
(Example)
Below this invention is described in greater detail with Example, but this invention is not restricted to these.

Test example 1 (histone deacetylase inhibition action).
(1) Preparation of [3H] acetyl histone
K 562 cell (10 power 8) was labelled with [3H] sodium n-butyrate, and histone was extracted according to process of Yoshida et al. (J. Biol. Chem., 265: 17174, 1990).

(2) Partial purification of histone deacetylase
Nucleus collected from K 562 cell (2.5 x 10 power 8) was extracted according to process of Yoshida et al. (J. Biol. Chem., 265: 17174, 1990), and partial purification of histone deacetylase was carried out from the extract thereof using Mono Q HR5/5 (Pharmacia company) by concentration gradient of NaCl of 0-1 M.

(3) Measurement of histone deacetylase inhibition activity
It was reacted for 10 minutes at 37 degrees in 50 µl Buffer A containing 100 µg/ml [3H] acetyl histone prepared in (1) and histone deacetylase fraction 2 µl prepared in (2) [composition: 5 mM potassium phosphate (pH 7.5), 5 % glycerol, 13 mM EDTA]. The reaction was stopped by addition of 2.5 N hydrochloric acid, and thereafter 550 µl ethyl acetate was added, vortex and centrifugation were carried out, and 400 µl ethyl acetate layer was collected in scintillation vial, 2 ml scintillator was added and radioactivity of [3H] acetic acid which was freed by reaction was measured. Measurement of histone deacetylase inhibition activity was determined by suitably diluting the test compound with buffer A after dissolution with DMSO, and adding to the reaction system, and the concentration of drug induced 50 % enzyme inhibition (IC50: µM) was determined. Below experimental results are shown in Table-2 (Table 15 - Table 17).

(0082)
(Table 15)

Table-2 histone deacetylase inhibition action

The compound number in Table -1 of Detailed Description	Activity value (IC50= μM).
1	2.01
4	9.13
5	4.20
8	4.23
9	7.01
11	18.50
12	6.89
13	0.87
14	3.22
15	3.72
16	2.88
17	2.66
18	2.43
19	1.94
20	5.11
22	2.46

(0083)
(Table 16)
Table -2 sequel (1).

The compound number in Table -1 of Detailed Description	Activity value (IC50= μM).
23	3.30
24	1.69
25	4.53
26	7.07
27	8.77
28	1.80
29	4. 85
30	5.04
31	10.43

32	24.30
33	3.01
34	4.11
36	6.89
38	12.25
39	1.42
40	1.75
41	3.72
42	2.99
43	3.27
44	5.40

(0084)

(Table 17)

Table -2 sequel (2).

The compound number in Table -1 of Detailed Description	Activity value (IC50= μM).
45	3.90
46	4.17
47	2.50
48	2.30
50	4.86
51	2.12
52	3.86
53	2.52
54	1.22
55	2.63
57	2.22
58	3.48
59	1.00
60	1.92
61	3.14
62	3.17
63	4.76

64	0.53
65	4.36
66	3.59
67	2.20
sodium butyrate	190

(0085)

Reference Example 1.

Synthesis of N-(2-aminophenyl)-4-[N-(pyridine-3-yl) methoxycarbonylamino methyl] benzamide (Table-1: compound number 14)

(1-1) Triethylamine 42 ml (300 mmol) was added to dichloromethane (450 ml) suspension of 4-aminomethyl benzoic acid 21 g (140 mmol). Dichloromethane (50 ml) solution of anhydrous trifluoroacetic acid 60 g (287 mmol) was added dropwise while being held under ice cooling to an internal temperature at 3-8 degrees and thereafter it was stirred for three hours. The reaction liquor was introduced into saturated sodium bicarbonate, and thereafter furthermore it was acidified with 10 % hydrochloric acid aqueous solution. Precipitated gel form precipitate was recovered by filtration, and dried, thereby 4-(N-trifluoroacetylamino methyl) benzoic acid 30 g (yield 87 %) was obtained as opaque solid.

1H NMR (270 M Hz, DMSO-d6) delta ppm: 4.47 (2H, d, J = 5.8 Hz), 7.39 (2H, d, J = 8.1 Hz), 7.93 (2H, d, J = 8.1 Hz), 10.08 (1H, t, J = 5.8 Hz), 12.95 (1H, br.s).

(0086)

(1-2) 1N sodium hydroxide aqueous solution (500 ml) was added to dioxane (1000 ml) solution of o-phenylenediamine 108 g (1.0 mol), and dioxane (500 ml) solution of di t-butoxy dicarbonate 218 g (1.1 mol) was added under ice cooling. It was stirred at room temperature for six hours thereafter was left to stand overnight. The solvent was concentrated to 1/2 vol and thereafter it was extracted with ethyl acetate. Organic layer was washed with saturated aqueous sodium chloride solution, dried and solvent was eliminated by distillation, next the obtained residue was refined with silica gel column chromatography (chloroform), and by washing obtained solid with ethyl ether N-t-butoxycarbonyl-o-phenylenediamine 68.4 g (yield 32 %) was obtained as white solid.

1H NMR (270 M Hz, CDCl3) delta ppm: 1.51 (9H, s), 3.75 (2H, s), 6.26 (1H, s), 6.77 (1H, d, J = 8.1 Hz), 6.79 (1H, dd, J = 7.3, 8.1 Hz), 7.00 (1H, dd, J = 7.3, 8.1 Hz), 7.27 (1H, d, J = 8.1 Hz).

(0087)
(1-3) Oxalyl chloride 21 g (165 mmol) was gradually added dropwise to dichloromethane (200 ml) suspension of 30 g (121 mmol) of the compound obtained in step (1-1) while being cooled with ice (internal temperature 10-15 degrees). During this, DMF was added sometimes (by 0.1 ml every about 2 ml of dropwise addition). After total quantity of dropwise addition, it was stirred till effervescence stopped, and thereafter it was stirred for one hour at 40 degrees. The solvent was eliminated by distillation, and thereafter azeotropic distillation was carried out with oxalyl chloride of excess toluene, and it was dissolved in dichloromethane (100 ml) once again. Acid chloride solution prepared beforehand was dropwise added to dichloromethane (100 ml)-pyridine (200 ml) solution of 22 g (110 mmol) of the compound obtained in Step (1-2), under ice cooling (internal temperature 7-9 degrees). On completion of the dropwise addition, it was warmed to room temperature, and thereafter it was left to stand overnight. Saturated aqueous sodium bicarbonate was added to the reaction mixture, and thereafter it was extracted with chloroform, and it was washed with saturated aqueous sodium chloride solution, dried and next the solvent was eliminated by distillation. Methanol-diisopropyl ether was added to obtained residue, and the precipitated solid was recovered by filtration, and dried, thereby N-[2-(N-t-butoxycarbonyl) aminophenyl]-4-(N-trifluoroacetylamino methyl) benzamide 28 g (yield 58 %) was obtained as pale yellow-coloured solid.

1H NMR (270 M Hz, DMSO-d6) delta ppm: 1.44 (9H, s), 4.48 (2H, d, J = 5.9 Hz), 7.12-7.23 (2H, m), 7.44 (2H, d, J = 8.1 Hz), 7.54 (2H, d, J = 8.1 Hz), 7.94 (2H, d, J = 8.1 Hz), 8.68 (1H, br.s), 9.83 (1H, s), 10.10 (1H, br, t, J = 5.9 Hz).

(0088)
(1-4) Potassium carbonate 4.7 g (34 mmol) was added to methanol (120 ml)-water (180 ml) suspension of 13 g (30 mmol) of the compound of step (1-3), and it was heated with stirring at 70 degrees for four hours. It was extracted with chloroform, and organic layer was washed with saturated aqueous sodium chloride solution, dried, and next the solvent was eliminated by distillation, and by drying 4-aminomethyl-N-[2-(N-t-butoxycarbonyl)

aminophenyl] benzamide 10.3 g (quantitative) was obtained as the pale yellow-coloured amorphous state solid.

1H NMR (270 M Hz, DMSO-d6) delta ppm: 3.80 (2H, s), 7.13-7.23 (2H, m), 7.48-7.58 (4H, m), 7.90 (2H, d, J = 8.1 Hz), 8.69 (1H, br.s), 9.77 (1H, br.s).

(0089)
(1-5) 3-pyridinemethanol 384 mg (3.5 mmol) was dissolved in 5 ml of dry THF, and N,N-carbonyldiimidazole 523 mg (3.2 mmol) was added at room temperature. It was stirred for one hour, and thereafter 6 ml dry THF solution of 1.0 g (2.9 mmol) of compound step (1-4) was added. It was left to stand at room temperature overnight, and next, chloroform 100 ml were added, and it was washed three times with water 20 ml. Thereafter, it was washed with saturated aqueous sodium chloride solution and next was dried with anhydrous magnesium sulphate. The solvent was eliminated by distillation under reduced pressure and was refined by silica gel column chromatography (chloroform : methanol = 30:1), and N-[2-(N-t-butoxycarbonyl) aminophenyl]-4-[N-(pyridine-3-yl) methoxycarbonylamino methyl] benzamide 1.2 g was obtained as the amorphous state solid (quantitative).

1H NMR (270 M Hz, CDCl3) delta ppm: 1.51 (9H, s), 4.45 (2H, d, J = 5.9 Hz), 5.16 (1H, s), 7.10-7.50 (7H, m), 7.70 (1H, d, J = 8.1 Hz), 7.80 (1H, d, J = 7.3 Hz), 7.93 (1H, d, J = 8.1 Hz), 8.57 (1H, d, J = 4.4 Hz), 8.63 (1H, s), 9.17 (1H, s).

(0090)
(1-6) 1.2 g (2.8 mmol) of the compound of step (1-5) was dissolved in 10 ml methanol. 4 N hydrochloric acid-dioxane solution 20 ml was added and it was stirred at room temperature for one hour 30 minutes. It was poured into dilute sodium hydroxide aqueous solution and thereafter it was extracted three times with 60 ml chloroform. It was washed twice with saturated aqueous sodium chloride solution, and next dried with anhydrous magnesium sulphate, concentrated, and the crystals of 0.88 g were obtained. Thereafter, it was re-crystallised from 16 ml ethanol, and N-(2-aminophenyl)-4-[N-(pyridine-3-yl) methoxycarbonylamino methyl] benzamide 668 mg (yield 73 %) was obtained.

Mp. 159-160 degC.

1H NMR (270 M Hz, DMSO-d6) delta ppm: 4.28 (2H, d, J = 5.9 Hz), 4.86 (2H, s), 5.10 (2H, s), 6.60 (1H, t, J = 7.3 Hz), 6.78 (1H, d, J = 7 Hz), 6.97 (1H, t, J = 7 Hz), 7.17 (1H, d, J = 8 Hz), 7.3-7.5 (3H, m), 7.78 (1H, d, J = 8 Hz), 7.93 (2H, d, J = 8 Hz), 8.53 (1H, d, J = 3.7 Hz), 8.59 (1H, s), 9.61 (1H, s).
IR (KBr) cm-1: 3295, 1648, 1541, 1508, 1457, 1309, 1183, 742.

(0091)
Advantages Afforded by this Invention.
Benzamide derivatives having histone deacetylase inhibition action of this invention are useful as therapy and/or improvement agents of diseases involving proliferation of cells, effect potentiation drug of gene therapy or immunosuppressive drug. They are highly effective as carcinostatic in particular and are effective for hematopoietic organ tumours and solid cancers.

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